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Severe Myositis Associated with *Sarcocystis* spp. Infection in 2 Dogs

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Background: Dogs are definitive hosts for numerous species of the intracellular protozoan parasite *Sarcocystis*. Reports of sarcocysts in muscles of dogs most often represent incidental findings.

Hypothesis/Objectives: To report the clinicopathologic, ultrastructural, and molecular findings in 2 dogs with myositis associated with *Sarcocystis* spp. infection, as well as the response to treatment with antiprotozoal drugs.

Animals: Two dogs with severe myositis in association with massive sarcocystosis.

Methods: Retrospective case review. Affected dogs were identified by a diagnostic laboratory. Attending clinicians were contacted, and the medical records reviewed. Immunostaining and electron microscopy were performed on muscle biopsies. Biopsies also were subjected to 18S rRNA gene PCR.

Results: Both dogs had fever, lymphopenia, thrombocytopenia, and increased serum alanine aminotransferase (ALT) activity when first evaluated. One dog developed hyperbilirubinemia. Subsequently, both dogs had increased serum creatine kinase activity and clinical signs of myositis, with reluctance to move, generalized pain, and muscle wasting. Histopathology of muscle biopsies showed severe inflammatory and necrotizing myopathy with numerous sarcocysts. Ultrastructural studies and 18S rRNA gene sequence results were consistent with infection with a *Sarcocystis* spp. other than *Sarcocystis neurona*. Both dogs initially were treated unsuccessfully with clindamycin and anti-inflammatory drugs. One dog died. The other dog subsequently responded to treatment with decoquinate.

Conclusions and Clinical Importance: *Sarcocystis* spp. infection should be included in the differential diagnosis for dogs that develop fever, thrombocytopenia, increased liver enzyme activities, and clinical and biochemical evidence of myositis. Although additional studies are required, decoquinate holds promise as an effective treatment for the disease.

Key words: Myopathy; *Neospora*; Neuromuscular; Protozoal; Sarcocystosis; *Toxoplasma*.

Sarcocystis spp. are intracellular protozoan parasites with an intermediate-definitive host life cycle. After ingestion of oocysts shed by the definitive host, organisms replicate asexually in the intermediate host, forming schizonts, usually in endothelial cells. Merozoites are subsequently released from the schizonts, and ultimately encyst, with the formation of a sarcocyst, which contains metrocytes that then develop into bradyzoites. The sarcocyst is the end stage of the parasite in the intermediate host, and is ingested by the carnivorous definitive host. Upon ingestion by the definitive host, bradyzoites are released from sarcocysts and transform into sexual stages (male and female gamonts) in the lamina propria of the small intestine. Fertilization results in formation of a zygote, and an oocyst wall is formed around the zygote. Oocysts sporulate in the lamina propria, are released into the intestinal lumen, and excreted in feces. Dogs are definitive hosts for numerous species of *Sarcocystis*.[1,2] There are reports of sarcocysts in muscles of dogs, mostly as incidental findings.[3–5] Myositis was associated with intramuscular sarcocysts in 1 dog from Canada.[6] Herein we report severe muscular sarcocystosis in 2 dogs from the United States, which was confirmed using ultrastructural and molecular methods. One of the dogs improved clinically after treatment with decoquinate.

Abbreviations:

ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
CK	creatine kinase
DAPI	4′,6-diamidino-2-phenylindole
DNA	deoxyribonucleic acid
MCV	mean corpuscular volume
MHC	major histocompatibility complex
PCR	polymerase chain reaction
rRNA	ribosomal ribonucleic acid
RR	reference range
Vp	villar protrusions

Materials and Methods

Medical Records

The medical records of 2 dogs diagnosed with sarcocystosis were reviewed. Attending veterinarians were contacted both by telephone and electronic mail to retrieve the medical records. Data obtained from the records included history, environment, and travel history, physical examination findings, laboratory data, diagnostic imaging results, treatment, and outcome.

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Light Microscopy, Immunofluorescence, and Electron Microscopy

Unfixed biopsies from proximal and distal limb muscles were collected under general anesthesia and shipped by an overnight service under refrigeration to the Comparative Neuromuscular Laboratory at the University of California, San Diego. Upon receipt, biopsies were flash frozen in isopentane, precooled in liquid nitrogen, and stored at −80°C until being further processed. Cryosections (8 μm) were stained or reacted with a standard histochemical panel, including hematoxylin and eosin, modified Gomori trichrome, periodic acid Schiff, ATPase at pH 9.8 or pre-incubated at pH 4.3, esterase, nicotinamide adenine dinucleotide-tetrazolium reductase, acid phosphatase, alkaline phosphatase, oil red O, and staphylococcal protein A-horseradish peroxidase, and then examined using light microscopy. Immunofluorescent antibody staining was performed on muscle cryosections using mouse monoclonal antibodies against canine leukocyte (CD3, CD4, CD8, CD11c) and major histocompatibility complex (MHC) Class I and Class II antigens.[7] A rabbit polyclonal antibody against α-sarcoglycan was used to outline the muscle sarcolemma, and 4′,6-diamidino-2-phenylindole (DAPI) stain[a] was used to highlight DNA in muscle nuclei.

Glutaraldehyde-fixed muscle biopsy specimens were postfixed in osmium tetroxide, and dehydrated in serial alcohol solutions and propylene oxide before being embedded in araldite resin. Thick sections (1 μm) were stained with toluidine blue for light microscopy, and ultrathin sections (60–90 nm) were stained with uranyl acetate and lead citrate for electron microscopy.

Nucleic Acid Extraction

DNA was extracted from thick muscle cryosections (20 μm) mounted on glass slides. Shavings from each slide were collected using disposable razor blades and added to 400 μL of phosphate-buffered saline and vortexed for 20 seconds. Total nucleic acid then was purified from 200 μL of the resulting suspension using an automated nucleic acid extraction system[b] according to the manufacturer's recommendations.

Real-Time PCR

Real-time PCR for *Neospora* spp., *Toxoplasma gondii*, and *Sarcocystis* spp. was performed at a commercial diagnostic laboratory.[c] The *Sarcocystis* spp. assay was designed using a conserved region of the 18S gene to detect *S. neurona*, *S. felis*, and *S. falcutula*. The *Neospora* spp. assay was designed using a conserved region of the ITS1 gene to detect *N. caninum* and *N. hughesi*. Positive and negative controls were included with each run. Each real-time PCR assay contained 20 μL of each primer and 4 μL of probe for each respective system, with a final volume of 220 μL for the primer-probe mix. The final concentration was 400 nmol/L for each primer and 80 nmol/L for the probe. PCR was performed using 1 μL genomic DNA, 4 μL water, and a commercially available PCR mastermix[d] containing 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 5 mmol/L $MgCl_2$, 2.5 mmol/L deoxynucleotide triphosphates, 0.625 U AmpliTaq Gold DNA polymerase per reaction, 0.25 U AmpErase uracil-N-glycosylase per reaction in a final volume of 12 μL. Samples were amplified in an automated fluorometer[e] and run with the *Sarcocystis*, *T. gondii*, *Neospora* spp., and eukaryotic 18S rRNA (NCBI X03205). Standard amplification conditions were used: 2 minutes at 50°C, 10 minutes at 95°C, 40 cycles of 15 seconds at 95°C, and 60 seconds at 60°C. Fluorescent signals were collected during the annealing temperature and cycle quantification values exported with a threshold of 0.1 and baseline values of 3–10.

Conventional PCR and Sequencing of 18S rRNA Genes

Extracted DNA was subjected to a broad-spectrum conventional *Sarcocystis* spp. PCR for amplification of a partial 18S rRNA gene sequence using previously described primers.[8] Each 25 μL reaction volume contained 1× reaction buffer, 1 U Amplitaq Gold polymerase,[a] 1.5 mmol/L $MgCl_2$, 400 nmol/L of each primer, 200 μmol/L of each dNTP, and 2.5 μL DNA template. Cycling conditions were 94°C for 3 minutes, followed by 40 cycles of amplification (40 seconds at 94°C, 60 seconds at 56°C, and 80 seconds at 72°C), and a final extension at 72°C for 5 minutes in a thermocycler.[f] PCR products were visualized after electrophoresis in an 2.5% agarose gel containing a nucleic acid stain.[g] Negative controls were included with each reaction. PCR products were sequenced directly in both directions.[h] The PCR products were ligated into a vector[i] and the plasmids transformed into competent cells.[j] Plasmids then were purified from cell lysates using a plasmid purification kit[k] and inserts were sequenced using automated methods. Sequences were compared with those deposited in GenBank using a basic local alignment search (BLAST).

Results

Case Descriptions

Case 1. An 11-year-old male neutered Golden Retriever from Cañon City, Colorado, was brought to a local veterinary clinic in January 2010 for a 1-day history of lethargy and anorexia. The dog had traveled to Nevada approximately 2 months before it became sick. Physical examination was unremarkable apart from a rectal temperature of 103.8°F. Body weight was 35.4 kg. An in-house complete blood count (CBC) showed a hematocrit of 49.9%, mean corpuscular volume (MCV) of 79.9 fL (reference range [RR], 60–77 fL), 4430 neutrophils/μL (RR, 2000–12000 cells/μL), lymphopenia (500 cells/μL; RR, 500–4900 cells/μL), and 67,000 platelets/μL (RR, 175–500 × 10^3 platelets/μL). Thrombocytopenia was verified by smear evaluation. A serum biochemistry panel showed increased activity of serum ALT (797 U/L; RR, 10–100 U/L) and serum alkaline phosphatase (ALP) (232 U/L, RR 23–212 U/L). Serum total bilirubin, albumin, cholesterol, and glucose concentrations were within RR. Serum activity of creatine kinase (CK) was not measured. A presumptive diagnosis of immune-mediated thrombocytopenia was made. Treatment with prednisone (1.1 mg/kg PO q12h), amoxicillin (14 mg/kg PO q12h for 30 days), famotidine (0.6 mg/kg PO q12h), sucralfate (28 mg/kg PO q8h for 14 days), and S-adenosylmethionine (6.3 mg/kg PO q24h) was initiated.

The dog's lethargy and anorexia subsided, but CBCs performed 13 and 31 days after initiating treatment showed persistent thrombocytopenia (85,000 platelets/μL and 81,000 platelets/μL, respectively). The prednisone dosage was decreased (1.1 mg/kg PO q24h), but over the following week the dog became lethargic

again, and weight loss (2 kg) was documented. A CBC showed neutrophilia (13365 cells/μL; RR, 3000–11500 cells/μL), 495 band neutrophils/μL (RR, 0), slightly toxic neutrophils, and clumped platelets. A biochemistry panel showed increased serum ALT activity (131 U/L). Serum CK activity was not measured. Thoracic radiographs were unremarkable. On day 38, the prednisone dosage was decreased further (20 mg PO q24h for 7 days, then q48h for 7 days) and tramadol was added (3.0 mg/kg PO q12h), but from day 51 the dog developed progressive pelvic limb weakness and anorexia.

Sixty-six days after onset of illness, the dog was examined by a board-certified veterinary neurologist. On physical examination, the dog was alert and interactive. Generalized muscle atrophy, a mild plantigrade stance in the pelvic limbs, stilted gait, and collapse with exercise were documented. Additional findings on neurologic examination were bilateral facial paresis, decreased conscious proprioception in all 4 limbs, delayed hopping reactions, and decreased segmental reflexes. Sciatic nerve sensation was decreased. The dog's rectal temperature fluctuated between 103.1 and 105.8°F over the next 24 hours. A CBC showed neutrophilia (20,114 cells/μL; RR, 2,060–10,600 cells/μL), monocytosis (904 cells/μL; RR, 0–840 cells/μL), nonregenerative anemia (34%) and a platelet count of 290,000/μL. A biochemistry panel showed hypoalbuminemia (2.5 g/dL; RR, 2.7–4.4 g/dL), increased serum activities of ALT (298 U/L; RR, 12–118 U/L), aspartate aminotransferase (AST) (197 U/L; RR, 15–66 U/L), CK (896 U/L; RR, 59–895 U/L), and hypomagnesemia (1.3 mEq/L; RR, 1.5–2.5 mEq/L). Treatment with ampicillin and enrofloxacin parenterally was initiated pending culture results. Aerobic and anaerobic bacterial blood cultures were negative. Aerobic bacterial urine culture yielded a broadly susceptible *Escherichia coli*. Urinalysis was not performed. Thoracic radiographs again showed no clinically relevant findings. Electromyography on day 67 showed spontaneous activity including increased insertional activity and fibrillation potentials in a generalized distribution. Motor nerve conduction velocities from the tibial and ulnar nerves were markedly decreased at 37 and 43 m/s, respectively (RRs, 68.2 ± 1.4 m/s and 58.9 ± 1.0 m/s, respectively).[9] Compound motor unit amplitudes were decreased with dispersion. Biopsies were collected from the deep digital flexor muscle and left common peroneal nerve and processed as described previously. Cerebrospinal fluid collected from the lumbar cistern showed a normal cell count and protein concentration of 62 mg/dL (RR, ≤30 mg/dL). Immunofluorescent antibody serology[l] for serum antibodies to *N. caninum*, *Babesia canis*, *Bartonella henselae*, *Bartonella vinsonii*, *Ehrlichia canis*, and *Rickettsia rickettsii* was negative. Results of serology using an in-house commercial diagnostic test[m] for *Borrelia burgdorferi*, *E. canis*, *Anaplasma* spp., and *Dirofilaria immitis* also were negative. The dog was discharged from the hospital on day 69. Medications were enrofloxacin (5 mg/kg PO q12h for 10 days) for the urinary tract infection, clindamycin (11 mg/kg PO q8h) pending results of *N. caninum* serology and cyclosporine (5.6 mg/kg PO q12h), and dexamethasone (0.06 mg/kg PO q24h) for a possible immune-mediated polyneuropathy.

Histopathology of the muscle biopsies showed severe protozoal myositis. On day 75, cyclosporine was discontinued and the dexamethasone dosage tapered (0.06 mg/kg PO q72h). Eighty-four days after onset of illness, the dog became anorexic and collapsed, and was brought to an emergency clinic. On physical examination, the dog was weak and panting. Ptyalism was noted. Rectal temperature was 105.3°F, pulse 146 beats/min, and the dog was estimated to be 5–7% dehydrated. Body weight was 29.5 kg. A PCV was 35% and the serum biochemistry panel was normal apart from mild hypernatremia (163 mEq/L; RR, 144–160 mEq/L). Serum CK activity was not measured. The dog was treated with fluids (lactated Ringer's solution, 65 mL/h, IV), meropitant citrate[n] (1 mg/kg, SC), dexamethasone sodium phosphate (0.07 mg/kg, IV, once), and clindamycin (12.7 mg/kg, IV, once), but died several hours after admission. Necropsy was not performed.

Case 2. A 4-year-old female spayed Rottweiler from Greenough, in rural Montana, was brought to a local veterinary clinic in March of 2010 with a 1-day history of lethargy, ptyalism, anorexia, watery diarrhea, and increased panting. Three days before the onset of clinical signs, the dog was suspected to have contacted, and possibly ingested, carrion. On physical examination, the dog weighed 46.1 kg, had a rectal temperature of 104.2°F, heart rate of 124 beats/min, respiratory rate of 22 breaths/min, had tacky mucous membranes, and abdominal pain. Radiographs of the thorax and abdomen were unremarkable. A CBC showed a hematocrit of 50.8% (RR, 37–55%), 7,100 neutrophils/μL (RR, 3,000–10,500 cells/μL), lymphopenia (200 cells/μL; RR, 1,000–4,000 cells/μL), monocytopenia (0 cells/μL; RR, 150–1,200 cells/μL), and thrombocytopenia (99,000 platelets/μL; RR 200–500 × 10^3/μL). The thrombocytopenia was confirmed by microscopic examination of a blood smear. A serum biochemistry panel, performed on serum that had been separated from the clot without delay after collection, showed increased serum ALT activity (176 U/L; RR, 10–100 U/L) and hypoglycemia (58 mg/dL; RR, 74–143 mg/dL). Serum CK activity was not measured. Treatment with metronidazole (22 mg/kg PO q12h) and amoxicillin (22 mg/kg PO q12h) was initiated without clinical improvement. Two days later, the dog was taken to a local emergency clinic. At that time, the dog's rectal temperature was 102.9°F, and it was treated with lactated Ringer's solution (250 mL/h IV).

A CBC performed the next day at the local clinic showed an HCT of 38.4%, 7,900 neutrophils/μL (RR, 3,000–10,500 cells/μL), 600 monocytes/μL (RR, 150–1,200 cells/μL), 300 lymphocytes/μL (RR, 1,000–4,000 cells/μL), and clumped platelets. A serum biochemistry panel showed increased serum activities of ALP

(2203 U/L; RR, 20–200 U/L), ALT (255 U/L; RR, 10–100 U/L), hyperbilirubinemia (3.5 mg/dL; RR, <0.4 mg/dL), and hypokalemia (3.3 mEq/L; RR 3.4–5.4 mEq/L). The dog was hospitalized and treated with enrofloxacin (2.2 mg/kg IV q12h), ampicillin (22 mg/kg IV q8h), and famotidine (0.43 mg/kg IV q12h). Abdominal ultrasound examination by a board-certified internist showed no clinically relevant findings. The next day, the dog began eating and was discharged from hospital. Treatment with amoxicillin and metronidazole was continued. Over the next week, the dog's appetite returned to normal and fever and ptyalism resolved. Although the dog's activity level improved, lethargy persisted.

At a recheck examination 28 days after the onset of illness, physical examination showed an stiff gait, reluctance to stand, and generalized muscle pain on palpation. A CBC and serum biochemistry panel was performed. The CBC showed an HCT of 44.5%. There were 4,700 neutrophils/μL (RR, 3,000–10,500 cells/μL), 300 monocytes/μL (RR, 150–1,200 cells/μL), 1,300 lymphocytes/μL (RR, 1,000–4,000 cells/μL), and 236,000 platelets/μL (RR 200–500 × 103/μL). Serum creatinine concentration was 1.1 mg/dL (RR, 0.4–1.4 mg/dL), and BUN concentration was 17 mg/dL (RR, 6–24 mg/dL). Serum ALT (294 U/L; (RR, 10–100 U/L) and AST (555 U/L; RR, 10–40 U/L) activities were increased, but serum ALP activity and total bilirubin concentration were within normal limits. Serum CK activity was increased (7,179 U/L; RR, 20–200 U/L). Serology for *Leptospira* spp. antibodies was negative.[o] Serology for *T. gondii* IgM antibody was negative.[p] Reciprocal antibody titers for *T. gondii* IgG antibody[p] and *Neospora caninum* antibodies[q] were positive at 64 and 40, respectively. Treatment with clindamycin (13 mg/kg PO q12h), prednisone (0.1 mg/kg PO q12h), and tramadol (2.2 mg/kg PO q6-8h) was initiated. This resulted in mild clinical improvement. Serum CK activity remained persistently increased (3,281 U/L on day 45 after onset of illness, and 5,406 U/L on day 50). Fifty-two days after the onset of illness, the dog was anesthetized and muscle biopsies were collected from the biceps femoris and triceps muscles, and histopathology of these tissues showed severe protozoal myositis.

Approximately 3 months after the onset of illness, protozoal serology was repeated. Serology for *T. gondii* was negative.[p] The reciprocal antibody titer for *N. caninum* was 800.[p] Treatment with clindamycin and tramadol was continued. An attempt was made to taper and discontinue the prednisone but the dog became painful again and so prednisone treatment was re-instituted. Serum CK activity remained increased almost 4 months after onset of illness (1,484 U/L), and the dog continued to exhibit generalized pain and muscle wasting.

Treatment with prednisone was discontinued and decoquinate (10–20 mg/kg PO q12h) was added. Over the next 3 months, the dog's muscle mass and energy level normalized and a 5 kg weight gain was recorded. Serum CK activity decreased to 474 U/L and the clindamycin was discontinued. The dog remains well at the time of writing, 9 months after initiating treatment with decoquinate.

Light Microscopy, Immunfluorescence, Electron Microscopy

Severe inflammatory and necrotizing myopathy was identified in both dogs after histopathologic examination of muscle biopsy sections. Numerous fibers contained encapsulated parasite cysts (Fig 1). Multifocal areas of mixed mononuclear cell infiltrations were present having an endomysial distribution with invasion of myofibers containing cysts. Immunofluorescence staining confirmed numerous $CD3^+$ T cells, $CD8^+$ T cells in greater numbers than $CD4^+$ T cells, and numerous $CD11c^+$ macrophage/dendritic cells (Fig 2). Surface labeling of parasite cysts within muscle fibers by the antibody against MHC class I antigen was striking (Fig 2). Histopathology of the nerve biopsy from case 1 showed moderate depletion of large myelinated fibers with a few remaining nerve fibers undergoing axonal degeneration. Foamy macrophages were evident within the endoneurium. Infectious organisms were not visualized in the examined sections of nerve.

Numerous sarcocysts were found in muscles of both dogs. Sarcocysts were up to 1,200 μm long and up to 75 μm wide, depending on the plane of section and orientation of myocytes. These sarcocysts were immature, septate, and contained metrocytes at the periphery, and differentiating bradyzoites centrally. The sarcocyst wall was thin (<1 μm thick) and lacked striations. In 1 μm toluidine blue sections, villar projections were seen on the cyst wall.

Four sarcocysts were examined ultrastructurally. The sarcocyst wall had short stubby villar protrusions

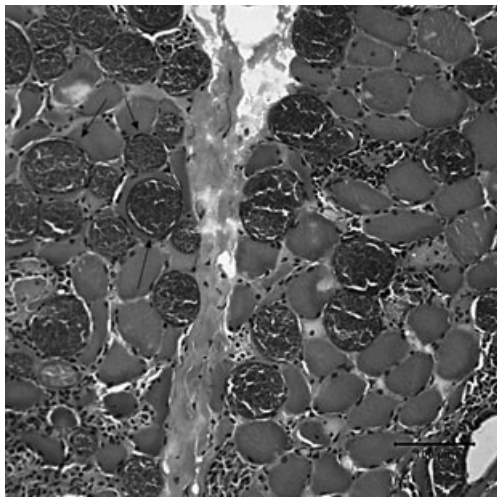

Fig 1. H&E stained cryosection of the biopsy from the deep digital flexor muscle of Dog 1 showing numerous myofibers containing large and encapsulated parasite cysts that were confirmed to be *Sarcocystis* (arrows identify examples of parasite cysts). Cellular infiltrates with an endomysial distribution with invasion of some myofibers also were observed (200× magnification).

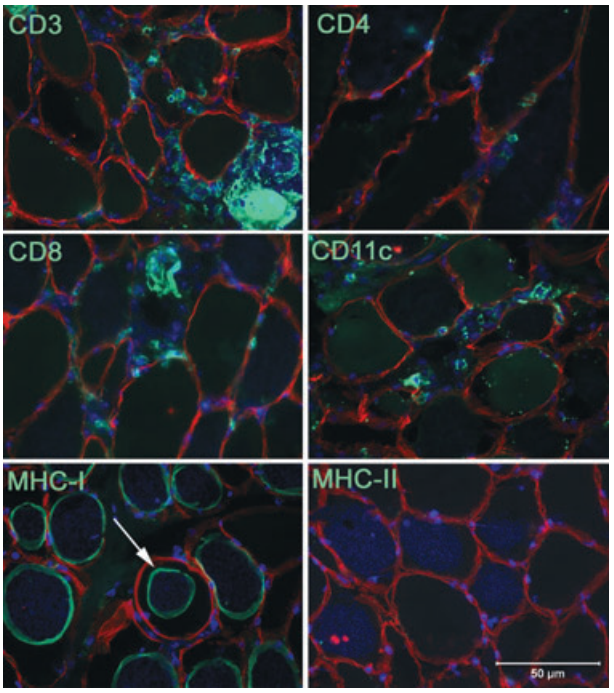

Fig 2. Immunofluorescent staining of cryosections from the triceps muscle of Dog 2 was performed using antibodies against canine leukocyte antigens CD3, CD4 and CD8 (T cell markers), CD11c (macrophage and dendritic cell marker) and against major histocompatibility complex (MHC)-class I and class II antigens. Leukocyte and MHC antibodies were labeled with fluorescein isothiocynate (green stain). An antibody against α-sarcoglycan was used to outline the muscle sarcolemma (red stain). Nuclei were identified by the 4′,6-diamidino-2-phenylindole stain. Note the surface labeling of parasite cysts within muscle fibers was highlighted with the antibody against MHC-class I (arrow).

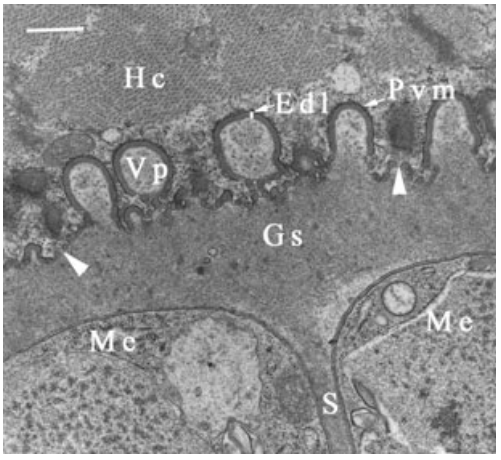

Fig 3. Transmission electron micrograph of a sarcocyst from a dog with myositis. The host cell (Hc) contains a sarcocyst, which contains metrocytes (Me). The sarcocyst wall consists of a villar protrusion membrane (Pvm) with short villar protrusions (Vp) and a homogenous ground substance (Gs) which is continued into the sarcocyst interior as septa (S). The Vp are lined by an electron dense layer (Edl), which is interrupted at irregular distances (arrowheads). Bar = 0.17 µm.

(Vp) on the entire wall (Fig 3). The Vp were up to 0.7 µm long and up to 0.4 µm wide, narrow at the base and expanded laterally (Fig 3). The wall of the sarcocyst (parasitophorous vacuolar membrane) had minute undulations. The membrane was lined by an electron dense layer that was interrupted at irregular distances. The interior of the Vp was homogenous, without microtubules. The homogenous granular substance layer was present underneath the Vp. The total sarcocyst wall, including the Vp and the granular substance layer, was approximately 1.0 µm thick in longitudinal section. The granular substance layer continued into the interior of the sarcocyst as septa.

PCR Results

Real-time PCR was negative for all pathogens tested. In both cases, conventional broad-spectrum PCR for *Sarcocystis* spp. resulted in the amplification of an approximately 823 bp product, which was cloned and sequenced (Genbank Accession Numbers JN256676 and JN256677). In both dogs, the resulting sequence had 99% identity to several *Sarcocystis* spp. from different hosts, including *Sarcocystis canis* from a dog,[10] a novel *Sarcocystis* spp. from a raccoon dog (*Nyctereutes procyonoides viverrinus*) from Japan,[11] *Sarcocystis arctosi* from a brown bear[12] and *Sarcocystis* spp. from wild avian species (Genbank Accession Numbers DQ146148, GU187943, EF564590, EU810398, GU253884).

Discussion

Although *Sarcocystis* myositis has been reported previously in a dog from Canada, this is the first time fulminant *Sarcocystis* myositis has been described in dogs from the United States, and the first report of successful treatment of the disease with decoquinate. Unlike a previous report from the United States,[13] the presence of severe myositis in the dogs reported here in association with massive numbers of sarcocysts implicates *Sarcocystis* spp. in disease causation. Ultrastructurally, the sarcocysts in the dogs reported herein appeared similar to those described in the dog from Canada.[6] In the dogs reported herein, molecular methods also were used to confirm infection with *Sarcocystis* spp. The sarcocysts in the dogs of this report were structurally distinct from those reported for *S. neurona*.[13] The villar protrusions on the sarcocyst wall in the present study were smaller than those of *S. neurona* and lacked central microtubules. The 18S PCR assay used herein did not permit identification of the species of *Sarcocystis* involved.

The *Sarcocystis* species found in these dogs have not been named or definitely identified. Infections with *Sarcocystis canis*, and *Sarcocystis neurona* have been reported in dogs.[14–18] *S. canis* was first described in 1991.[15] The complete life cycle of *S. canis* is unknown. *S. canis* was named to draw attention to the severe

hepatitis it causes in dogs and other animals; the only stage known is the schizont.[15,17] Encephalitis, dermatitis, and pneumonia also have been reported in dogs infected with *S. canis*, with most reports involving puppies.[15,16,18] *Sarcocystis neurona* is an unusual species with opossums as definitive hosts and several other animal species, including dogs, cats, marine mammals, and horses, as intermediate or aberrant hosts.[10,19] There are numerous reports of severe *S. neurona*-like infections in animals, usually with central nervous system signs associated with schizont stage.[14,19,20] Immature *S. neurona*–like sarcocysts were reported in the muscle from a dog with chronic myositis, but the cause of myositis was not determined because schizonts also were present in the muscle interstitium.[13] In addition, the sarcocyst density was considerably lower than in the dogs reported herein; only 30 individual cysts were observed in 60 sections taken from 5 different parts of the body in the dog infected with the *S. neurona*-like organism.[13] Sarcocysts also have been identified as incidental findings in cardiac and skeletal muscle of dogs from India,[3] Kenya,[21] Georgia[22] and Alabama,[5] in the absence of associated inflammation or clinical signs of myositis.

As described for the dog with *Sarcocystis* myositis in Canada,[6] both the dogs in this report had increased serum liver enzyme activities in association with illness. Increased activities of serum ALT and AST have been reported in association with muscle injury in dogs[23] and also in human patients.[24] Because the increases in serum ALT and AST activities were never documented to occur in association with normal serum CK activity, we could not definitively conclude that the increases in liver enzyme activities resulted from hepatic injury. However, one of the dogs in this report as well as the dog from Canada also developed moderate to marked hyperbilirubinemia,[6] which would not be expected to result from muscle injury alone. Although unconfirmed, it is possible that hepatic injury in these dogs may have been associated with hepatic schizogony preceding sarcocyst formation, as observed in dogs with hepatitis associated with *S. canis* infection.[15,17] Of interest, a puppy with fatal hepatitis associated with *S. canis* infection also had persistent mild increases in serum CK activity,[17] as well as moderate thrombocytopenia, which also was documented in both dogs in this report.

Serum antibodies to *N. caninum* were detected in Dog 2 but not Dog 1 of this report. Serum antibodies to *N. caninum* also were detected in the Canadian dog.[6] The cysts in that report did not react to *N. caninum* antibodies prepared in a rabbit, and it was speculated that the positive *N. caninum* titer might have reflected previous exposure to *N. caninum* without active infection. The same may be true for Dog 2 reported herein. Although an apparent change in titer to *N. caninum* occurred in this dog, acute and convalescent serology was performed at 2 different laboratories, and the results are not comparable.

Reactivation of bradyzoites from sarcocysts with additional sarcocyst formation has not been demonstrated for any species of *Sarcocystis*.[2] Unlike *T. gondii*, bradyzoites released from ruptured sarcocysts do not initiate new infections. Therefore, given the massive numbers of sarcocysts present, it is likely that the dogs in this report ingested large numbers of oocysts. Similarly, it is unlikely that the immunosuppressive drug treatment in Dog 1 contributed to the number of cysts present, given that the immunosuppressive drug treatment was initiated after the onset of clinical signs. Immunosuppression has been associated with clinical sarcocystosis in other animal species[25,26] and it is possible, although unconfirmed, that the pharmacologic immunosuppression may have contributed to the rapid progression of disease in Dog 1 through unrestrained parasite replication within sarcocysts and sarcocyst rupture, with an associated inflammatory response. Nevertheless, the strong labeling of the cyst surface with an antibody against MHC Class I indicated the presence of an immune response against the organism. The resemblance of the clinical signs and laboratory findings to immune-mediated thrombocytopenia and immune-mediated polymyositis suggests that practitioners should consider disseminated protozoal disease in dogs presenting with these findings before initiation of immunosuppressive drug treatment.

The dog from Canada with *Sarcocystis* spp. myositis experienced a complete recovery after treatment with clindamycin.[6] In contrast, use of clindamycin alone for 4 months was not associated with substantial clinical improvement in the second dog reported herein. In contrast, initiation of treatment with decoquinate was associated with almost complete resolution of clinical and biochemical abnormalities. Decoquinate is a quinolone coccidiostat that has been used successfully to treat canine hepatozoonosis.[27]

In summary, infection with *Sarcocystis* spp. should be considered in dogs living in North America that develop fever, thrombocytopenia, an increased CK activity, and muscle stiffness and atrophy. The concurrent presence of increased liver enzyme activities also may support the diagnosis. Additional research is required to evaluate the efficacy of decoquinate for treatment of this disease, but the clinical improvement seen in Dog 2 suggests it may be of value. Additional studies are needed to determine the species of *Sarcocystis* spp. involved and to better understand the life cycle of this parasite.

Footnotes

[a]Invitrogen, Carlsbad, CA
[b]QiaX-tractor; Qiagen, Valencia, CA
[c]Lucy Whittier TaqMan Laboratory, Davis, CA
[d]TaqMan Universal PCR Mastermix; Applied Biosystems
[e]ABI PRISM 7900 HTA FAST, Applied Biosystems
[f]Dyad, MJ Research Inc., Waltham, MA
[g]GelStar, Lonza, Rockland, ME
[h]Davis Sequencing, Davis, CA
[i]pGEM-T Easy, Promega, Madison, WI
[j]XL1 Blue, Stratagene, La Jolla, CA

[k]UltraClean; MO BIO Laboratories, Carlsbad, CA
[l]Vector-Borne Diagnostic Laboratory, North Carolina State University College of Veterinary Medicine, Raleigh, NC
[m]SNAP 4Dx; IDEXX Laboratories, Westbrook, ME
[n]Cerenia; Pfizer Animal Health, Madison, WI
[o]Michigan State University, Diagnostic Center for Population and Animal Health, East Lansing, MI
[p]Colorado State University, Veterinary Diagnostic Laboratories, Fort Collins, CO
[q]Texas Veterinary Medical Diagnostic Laboratories, College Station, TX

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